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(57) Abstract

Hollow (i.e. gas- or vapour-filled) microcapsules, for example of albumin, are prepared by forming a shell around a solid or liquid core and subsequently removing the core. The core may be a volatile oil such as perfluorohexane. The shell may be made by simple or complex coacervation, oil/water/oil double emulsion, or MSIEP (minimisation of solubility at isoelectric point) methods, followed by chemical or heat hardening to render it water-insoluble. When the double emulsion method is used, the microcapsules have a honeycomb appearance with multiple gas-filled chambers. The microcapsules can be used for echocardiography.

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DIAGNOSTIC AID

The present invention relates to diagnostic aids and, more particularly, to echogenic materials for echocardiography and other purposes.

As is known from EP-A-0 324 938, air-filled albumin microbubbles of about 1-10 μm can be injected into the bloodstream and will reflect ultrasonic radiation in such a way as to yield diagnostically-useful images of the heart and other internal organs. These microbubbles are formed by sonicating viscous aqueous albumin solutions at 5000 - 30,000 Hz. The resulting microbubbles are heat-denatured to make the albumin water-insoluble.

We have now devised an improved process for preparing hollow microcapsules, rather than microbubbles, which has been found to give a high yield of particles which are better suited for echocardiography.

One aspect of the invention provides a process for preparing gas-containing microcapsules comprising forming water-dispersible (preferably proteinaceous) microcapsules having a liquid or solid core and removing at least some of the said liquid or solid to create a microcapsule containing a gas.

Forming non-proteinaceous microcapsules in this way has previously been proposed in GB-A-1 288 583 for use in paints. The polymers used in GB-A-1 288 583 were insoluble polymers like polystyrene. There was no suggestion of their use as injectable compositions for echocardiography, whereas the compositions of the present invention, at least when used for such a purpose, are biocompatible, biodegradable and non-immunogenic.

A. Kondo in "Microcapsule Processing and Technology" (Marcel Dekker Inc, New York, 1979) suggests forming hollow capsules using a low boiling point solvent as the core in an in-liquid drying process (page 109) and oil-containing gelatin capsules from which the oil is not subsequently removed. US-A-4 173 488, US-A-3 781 230 and US-A-4 089 800 disclose the use of hydrophobic resins and hydrophobic starches to coat the oil droplets in an oil-in-water emulsion and subsequently form microcapsules. None of these documents mentions using the microcapsules for echocardiography and none mentions the use of albumin. EP-A-0 327 490 discloses the use of synthetic polymers to surround gas bubbles in a liquid medium and subsequently form microcapsules for echocardiography. This is a different process from that of the present invention and the prior specification does not mention using proteins.

The core in the process of the present invention is preferably a water-immiscible oil and is preferably also relatively volatile so that it can be evaporated after the microcapsules have been formed, in other words during or after the hardening of the wall. This is what we mean by "relatively volatile". More specifically, any inert oil, preferably a perfluoro compound, having a boiling point of 20-100°C, preferably 40-90°C and more preferably 50-80°C is generally suitable. Perfluorohexane, perfluoroheptane, perfluoromethylcyclohexane, cyclopentane, hexane, 2-methylpentane, 3-methylpentane, 2,2, dimethylbutane, 2,3, dimethylbutane, 1-chloropropane, 2-chloro-2-methyl propane, chloroform, methylene chloride, 1,1 dichloroethane and bromoethane are all suitable. More than one core can be provided in each microcapsule. A solid core, such as ammonium carbonate, may be used, followed by sublimation or removal with a solvent.

The process for the production of hollow microcapsules may be any of those generally known as simple coacervation, complex coacervation, MSIEP (minimisation of solubility at isoelectric point) and double emulsion, but is preferably the latter. Interfacial polymerisation may be used for some wall-forming materials, although not for proteinaceous materials.

Any suitable wall-forming material may be used which is (i) dispersible (preferably soluble) in water, (ii) capable of being rendered water-insoluble once the microcapsules are made and (iii) physiologically non-toxic and non-immunogenic, at least in the conditions of use. Materials which are biodegradable in the patient following administration are preferred. Proteinaceous materials are preferred and serum albumin is generally suitable. The term "proteinaceous" is used herein to describe proteins, naturally-occurring and synthetic polypeptides and fragments of proteins and polypeptides. For human use, human serum albumin (HSA) is preferred. This can be isolated from serum by known techniques or manufactured by recombinant DNA techniques such as are disclosed in EP-A-201 239 and EP-A-286 424. Analogues and fragments of HSA can be used, such as are disclosed in EP-A-322-094. In this specification, the term "albumin" is used to cover all of these compounds. Other materials include gelatin, hydroxyethyl starch, starch and dextran. The properties of some materials, such as albumin, may be modified by the presence of an added non-ionic surfactant, such as is described by Omotosho *et al* as interfacial complexation (1986 *J. Pharm. Pharmacol.* **38**, 865-870). The materials are chemically or thermally denatured, to render them insoluble, after the microcapsules have been formed.

The (preferably proteinaceous) material can be made water-insoluble by chemical cross-linking, denaturation (for example with heat), chelating or grafting.

The microcapsules of the invention are filled with a gas or vapour, which may be air or any other true gas but is often a mixture of air and the vapour from the volatile oil. In this specification, the term "air-filled" is loosely used to cover pure air, any other gas, any vapour or mixtures thereof.

The microcapsules which are formed are preferably from 0.1 to 500 μm in diameter. For use in echocardiography, a range of 1.0 to 10 μm or 2.0 to 8 μm is especially suitable. Such sizes may be achieved by appropriately selecting the process parameters and/or by separating out, for example by wet micro-sieving or air elutriation, the desired size from the resulting microcapsules. Since a range of sizes will usually result, the figures in this specification refer to 90% of the population by weight. The size range can be measured with a light microscope or by using known apparatus such as the Coulter Counter and known methods such as those disclosed in Morris & Warburton, *J. Pharm. Pharmacol.* 36, 73-76 (1984).

At least in the case of the double emulsion methods, a multi-chamber microcapsule results, resembling a honeycomb or the type of confectionery sold in the UK under the registered trademark

"Malteser". This is a preferred product. There may be from two to several hundred chambers in each microcapsule, preferably at least 10.

The final product is typically obtained in the form of a suspension which may be washed, sterilised and used. In at least some cases, however, the microcapsules can be freeze-dried without collapsing and stored as a free-flowing powder for future use.

The air-filled microcapsules may be used in echocardiography and other ultrasonic imaging techniques in ways known in the art (see, for example, EP-A-0 324 938, US-A-4 276 885 and US-A-4 572 203, all incorporated by reference), in nasal and lung delivery systems for drugs (when prepared as powder, rather than suspensions) and as opacifiers or reflectivity enhancers in cosmetics.

The air-filled microcapsules themselves (especially the multi-chamber capsules) and their uses, particularly as echogenic materials in diagnostic procedures, form further aspects of the invention.

Examples of the invention will now be given with reference to the accompanying figures, in which:

Figures 1 and 2 are views from above and one side of respective stirring paddles;

Figure 3 is a vertical section of a mixing vessel in which the paddles operate; and

Figures 4 and 5 are respective scanning electron micrographs of microcapsules prepared in accordance with the invention using the double emulsion method.

EXAMPLE 1: SIMPLE COACERVATION

This method was adapted from one described in US Patent 2,800,458 (1957), for the production of carbonless copying paper. Various volatile oils were homogenised using a hand homogeniser (room temp., 15 mins) with 20 ml of a 10% aqueous solution of albumin, to form an o/w emulsion. Initially 1 ml of the oil perfluoro-1,3-dimethyl cyclohexane, which has a boiling point of 101-102°C, was used. Other oils such as dichloromethane (B.P. 39.8 - 40°C) and perfluorohexane (B.P. 58 - 60°C) were later employed. A dehydrating agent (isopropanol (6 ml) or a salt eg 6 ml of 20% sodium sulphate can be used) was then added over 10 mins, to induce coacervation, or concentration of the albumin around the droplets of volatile oil, and the product was stirred for 1 hour at 1233 rpm. A surfactant (Span 80 (sorbitan mono-oleate); 0.2 ml; was added

after coacervation and before cross-linking to prevent agglomeration of the microcapsules following cross-linking. The albumin was cross-linked using glutaraldehyde (0.2 ml) and excess reagent was inactivated with sodium metabisulphite (0.4 ml of 12% aqueous solution), which reacts with free aldehyde groups. The suspension of microcapsules obtained was stored in a desiccator at 5°C. The capsules were sized using a Malvern 3600 particle sizer.

Using this method, microcapsules were produced. Most were much smaller than 5 μm in diameter. By reducing the stirring speed from 1233 to 874 rpm, using perfluoro-1,2-dimethyl cyclohexane as volatile oil and Span 80 as surfactant, the yield of microcapsules in the size range 2 - 8 μm was increased but the range was also broader. When the surfactant was changed to Pluronic F68, the proportion of microcapsules in the desired size range increased to 71.7%; however, the range was still broad. (Pluronic F68 is the trade designation for poloxamer 188 (poloxalkol), a block copolymer of polyoxyethylene and polyoxypropylene (CAS-9003-11-6).) The nature of the volatile oil was also found to affect the particle size, with dichloromethane and perfluorohexane both producing smaller microcapsules than perfluoro-1,3-dimethyl cyclohexane, under the same conditions.

EXAMPLE 2: SIMPLE COACERVATION

The basic method of Example 1 was followed. 1 ml of perfluorohexane was homogenised into 10 ml of a 10% aqueous albumin solution in 30 sec using a Silverson homogeniser at 6800 rpm, following by stirring at 1370 rpm for 15 mins, at room temperature. The isopropanol was added as before but this step was followed by stirring for 1.5 hours at 1370 rpm. Similarly, the additions of Span 80 and glutaraldehyde were each followed by 15 min of stirring at 1370 rpm instead of 1233 rpm. Excess glutaraldehyde was removed with ethanolamine (0.8 ml) and the final stirring was at 1370 rpm for 15 min. The product was obtained as a suspension of relatively uniform microcapsules in the desired range of 2 - 8 μm.

EXAMPLE 3: DOUBLE EMULSION METHOD

A primary o/w emulsion was produced by homogenising a volatile oil (perfluoro-1,3 dimethyl cyclohexane) with a solution of HSA, as in Example 1. This emulsion was then re-emulsified into olive oil to produce an o/w/o emulsion, with the volatile oil as the inner oil phase. After addition of a surfactant, Pluronic F68, to prevent agglomeration of the particles, glutaraldehyde was added to cross-link the albumin. The excess glutaraldehyde was then inactivated using sodium metabisulphite. The resulting microcapsules were separated by centrifugation and washed with

petroleum ether and acetone, to remove the olive oil. After drying overnight in a desiccator at room temperature, the microcapsules were collected as a dry powder. Details of the method are as follows.

0.5 ml perfluoro-1,3-methylcyclohexane was homogenised into 1 ml of 20% aqueous HSA solution over 5 min at 6800 rpm. This o/w emulsion was poured into 25 ml of previously stirred olive oil and stirred at room temperature for 15 min at 1233 rpm. 0.4 ml of 10% Pluronic F68 was added and stirred for 15 min at 1233 rpm. 0.2 ml of glutaraldehyde was added and stirred as before. 0.4 ml of 12% aq. sodium metabisulphite added and stirred as before. The product was centrifuged at 3000 rpm for 20 min and washed etc as above.

Hollow microcapsules of 20-100 μm were obtained.

EXAMPLE 4: DOUBLE EMULSION

15 ml of perfluorohexane (Aldrich, UK) was added to 30 ml of 10% (w/v) HSA solution (fraction V; Sigma, UK) and emulsified with a Microfluidizer (Microfluidics Corporation, Newton, Mass, USA) in a continuous process for 45 seconds to form an o/w emulsion. 1.5 ml of this emulsion was then added to 50 ml of soya oil (Sainsbury, UK) at 22°C and emulsified with a homogenizer (Silverson, UK) at 6800 rpm for 5 minutes. The resultant o/w/o

emulsion was transferred to an oil bath and then heated at 120°C for 30 minutes, while the mixture was stirred using a mechanical stirrer (Heidolph RZR-1) at 874 rpm.

The mixture was allowed to cool to 25°C. Once this temperature had been reached, 20 ml of petroleum ether (May & Baker, UK) was added to the microsphere-soya oil suspension. This mixture was centrifuged at 3000 rpm for 20 minutes. The supernatant was decanted and the microspheres were washed with 40 ml of petroleum ether, centrifuged, decanted; washed again with petroleum ether and finally with ethanol.

Differing conditions were tried, for example using a mechanical stirrer or a homogenizer for the o/w/o emulsion; 1, 2 or 3% o/w emulsion in the soya oil; olive instead of soya oil; 874, 1250 or 2000 rpm stirring speed; type of paddle; 16.7 or 33.3% volatile oil; non-volatile oil (n-dodecane) instead of volatile oil; 5, 10 or 20% HSA; and 0, 1 or 10% lecithin as a surfactant in the primary emulsion.

Preferred conditions included: using a homogenizer to prepare the primary emulsion using 1% or 2% o/w emulsion in the soya oil; using soya instead of olive oil; using a vertical paddle as shown in Figure 2 at 2000 rpm, optionally with baffles in the mixing vessel; using volatile or non-volatile oil at 33.3%; absence of lecithin.

All particles were sized using a laser diffraction technique (Malvern Particle Sizer Type 2600 D, Malvern Instrument, UK). The particles were resuspended in water and sonicated (Soniprobe 7532B, Dawe, UK) for 2 minutes at 60 W before sizing. The shape and possible agglomeration of the particles were studied using a light microscope (Optiphot, Nikon, Japan). The product was a free flowing powder of size range 2-20 μm.

EXAMPLE 5: DOUBLE EMULSION

The method of Example 4 was adapted as follows to produce a particularly satisfactory result. 10 ml of perfuorohexane was emulsified into 20 ml of 10% aq. HSA with Microfluidiser, circulating the liquid three times at 60 - 90,000 kPa. 1 ml of o/w emulsion was poured into 50 ml soya oil and homogenised with the Silverson blender for 5 min at 6800 rpm. The albumin was cross-linked by heating to about 120°C in an oil bath (15 min equilibration; 30 min heating) whilst paddle stirring at 2000 rpm and then cooled to room temperature, followed by paddle stirring at 2000 rpm whilst adding 20 ml petroleum ether. The product was paddle stirred at 2000 rpm for 2 min, centrifuged at 3000 rpm for 20 min, decanted, washed twice with ether (20 ml) and once with ethanol (20 ml), shaken, centrifuged and decanted. Finally, the product was freeze-dried.

The product was a free-flowing powder of 2-10 μm microcapsules.

EXAMPLE 6: MINIMIZATION SOLUBILITY AT ISOELECTRIC POWER (MSIEP)

A method was developed for producing albumin microcapsules by the MSIEP technique. The preliminary results obtained by this method are discussed below. The MSIEP method uses elements of both complex coacervation and simple (o/w) emulsion techniques.

1 ml of perfluorohexane was emulsified with 10 ml of 10% HSA solution with a Silverson or microfluidiser to give a primary o/w emulsion to which 10 ml of 5% or 10% aq. HSA solution (pH 6.65) was added at room temperature, whilst stirring at 800 rpm. By decreasing the pH of the mixture to <4.7 with 1M HCl, whilst stirring at 800 rpm, the albumin in the mixture comes out of solution at the isoelectric point and forms a coating around the emulsion droplets due to neutralization of charges on the surface of the albumin in the emulsion and the solution. The albumin coating can then be cross-linked by heat or a chemical method(s) as described above. If glutaraldehyde is used to cross-link the albumin (typically 1 ml of 25% solution) then excess glutaraldehyde can be removed with 2 ml of ethanolamine (free base).

EXAMPLE 7: COMPLEX COACERVATION

This is a modification of a known method for the preparation of non-hollow microcapsules (see for example US 4808408, incorporated herein by reference). It relies upon the interaction of polymers in solution carrying opposite charge. Albumin (isoelectric point 4.7) which will carry a negative charge at pH 6.3 may be combined with a gelatin (isoelectric point in the range 7 to 9), which will carry a positive charge at this pH. Other suitable polymer mixtures may be used provided one is negative and the other positive at the chosen pH. For example, at low pH (4.0) albumin will be positively charged and may be made to interact with a negatively charged polymer (eg sodium alginate) or type B gelatin (isoelectric point 4.7-5.0). The choice of the complexing material for albumin will be based upon toxicity considerations.

More specifically, an oil-in-water emulsion was formed from 10 ml of 10% HSA (pH 6.3) and 1 ml perfluorohexane by Silverson blending for 5 mins at 6800 rpm, and then stirred at 638 rpm for 10 mins at 45°C. 10 ml of 10% gelatin type A (pH 5.9) was slowly added to give a preparation at pH 6.3 which was stirred as before. 0.4 ml of Span 80 was added and the mixture was stirred as before, following which 0.1 ml of 37% aqueous formaldehyde (cross-linker) was added and the mixture was stirred as before. Finally, 0.2 ml of 12% w/v sodium

metabisulphite was added to quench the formaldehyde and the mixture was stirred as before. A suspension of 2-50 μm microspheres was obtained.

The microcapsules may be filtered, washed and dried.

EXAMPLE 8: DOUBLE EMULSION

Materials: 25% Human Serum Albumin (HSA) Alpha Therapeutic Corporation, Los Angeles, USA. Soya oil (edible grade) J. Sainsbury plc. Petroleum ether Bpt 60-90C°(AR) Fisons, Loughborough, UK. Ethanol Absolute (AR) Fisons, Loughborough, UK. Acetone (AR) Fisons, Loughborough, UK. Fluorophore Filters (0.5 μm pore size), Millipore Filters.

Volatile oils: Perfluorohexane (99%) Aldrich Chemicals Ltd, UK. Perfluorodecaline Rhone Poulenc ISC Division, Avonmouth, Bristol. Perfluorodimethylcyclohexane Aldrich Chemicals, UK. Perfluoromethylcyclohexane Aldrich Chemicals, UK.

Instrumentation: Microfluidiser 120E, Christison Scientific Equipment Ltd, Gateshead, UK. Silverson homogeniser L4R, NorthernMedia, Nottingham. Homogeniser heads: Coarse head (2 mm circular pores), Fine head (1 mm circular pores), Heidolph stirrer ST1. Stirrer heads: 6-blade turbine, 4-blade paddle, 4-blade rotor.

Method: Emulsion formulations: Primary emulsion, 20 ml HSA (10%), 10 ml volatile oil.

Secondary emulsion: 15 ml primary emulsion, 500 ml Soya oil.

Preparation of the primary emulsion. The HSA and the volatile oil, which was any of those listed above or a combination of two in varying proportions, were mixed. The mixture was then emulsified using the microfluidiser or the Silverston homogeniser. The Microfluidiser was used at an operating pressure of 5.5-9.7 x 10^7 N/m^2 (8000-14000 pounds per square inch). The homogeniser was operated at 5000-9000 revolutions per minutes (rpm). The emulsion was manufactured in the microfluidiser either with or without the cooling coil. It was processed through 1-4 cycles. With the homogeniser, the volumes of the formulation were scaled up by a factor of 4 to make up the minimum homogenisation volume. The emulsion was then homogenised for 1-4 minutes. The emulsion was used as soon as possible after manufacture or stored at 4°C for use after a few hours.

Prepartion of the secondary emulsion and heat-fixing. 15 ml of the primary emulsion was added to 500 ml of soya oil and homogenised at 5500 rpm for 3 minutes. The emulsion was then transferred to a heated oil-bath and heated at the rate of 1 or 2°C per minute. The emulsion was stirred continuously during

heating using one of the stirrer heads described above at speeds which varied between 600-1500 rpm. The emulsion was heated to 120°C and held there for 20 minutes. The emulsion was then allowed to cool at room temperature and the microcapsules were harvested.

About 100 ml of petroleum ether was added to the fixed emulsion and stirred. The mixture was then centrifuged at 4500 rpm for 20 minutes. The supernatant was discarded and the pellet collected. The pellet was then resuspended in ether and passed vacuum-filtered through a 0.5 μm pore Fluorophore filter. The pellet was washed with ether, ethanol and acetone successively. The dry filter-cake was then allowed to dry at ambient temperature in a desiccator and then freeze-dried and stored at room temperature.

The size of the pores within the microcapsules depended on the volatile oil and the method of homogenisation used in the manufacture of the primary emulsion. Perfluorodecalin in conjunction with the Microfluidiser tended to produce microcapsules with an interior having a plurality of hollow spaces, resembling a "Malteser" sweet. ("Malteser" is a registered trademark.) Perfluorohexane emulsions made using the Microfluidiser tended to be solid while perfluorohexane emulsions made using the Silverson homogeniser were thin-walled microspheres with 5-10 pores per microcapsule. Flow charts for

two methods are given in Tables 1 and 2 below. Scanning electron micrographs of the product are shown in Figures 4 and 5. In Figure 4, the microcapsules have been prepared as follows: 1° **emulsion:** 60 ml 10% HSA, 30 ml Perfluorodecalin, microfluidised at 4.8 x 10^7 N/m^2 (7000 psi), 4 cycles, and homogenised at 6500 rpm for 5 minutes. 2° **emulsion:** 15 ml of the 1° emulsion was added to 500 ml soya oil and homogenised at 5500 rpm for 3 mins. The emulsion was stirred at 1500 rpm using a 6-blade stirrer head. The sample was freeze-dried before microscopy. In Figure 5, the microcapsules have been prepared as follows: 1° **emulsion:** 20 ml 10% HSA, 10 ml Perfluorodecalin, microfluidised at 9.7 x 10^7 N/m^2 (14000 psi), 4 cycles. 2° **emulsion:** 15 ml of the 1° emulsion was added to 500 ml soya oil and homogenised at 5500 rpm for 3 mins. The emulsion was stirred at 3000 rpm using a 6-blade stirrer head. The sample was freeze-dried before microscopy.

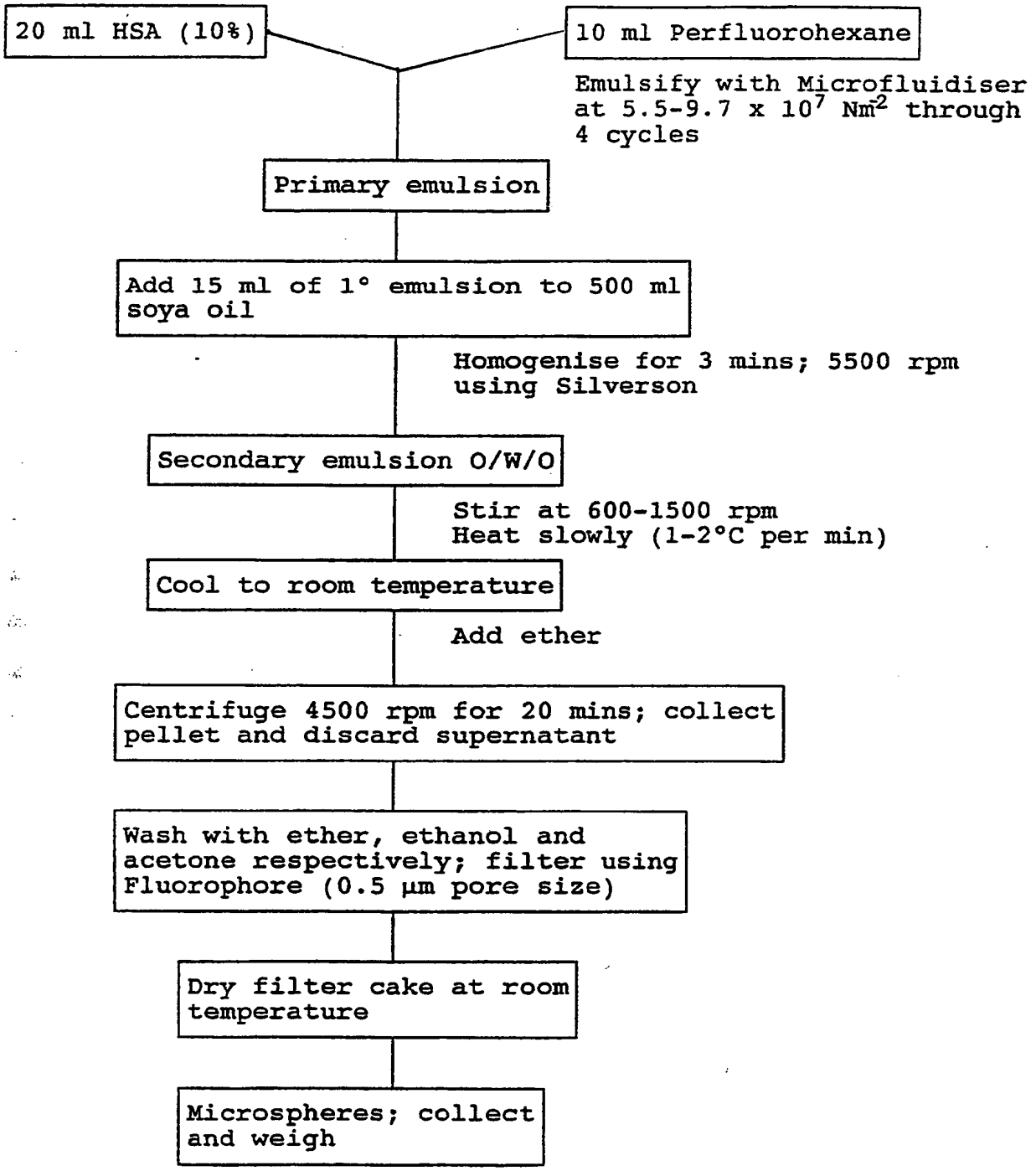

Table 1

Scaled-up process for the manufacture of HSA microcapsules using the double emulsion method (A).

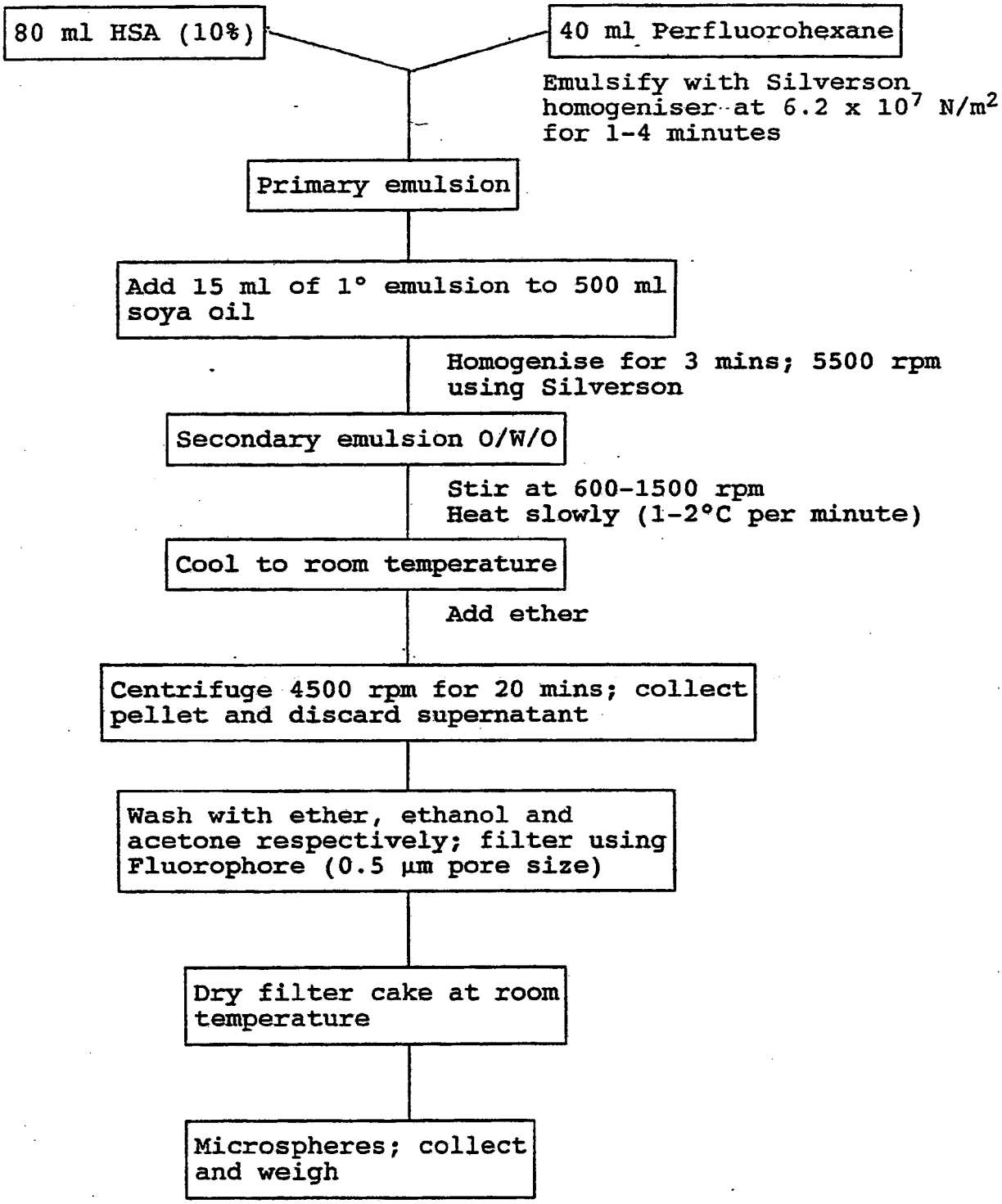

Table 2

Scaled-up process for the manufacture of HSA microcapsules using the double emulsion method (B).

GENERAL

The whole process of the invention can advantageously be carried out aseptically, starting with raw materials filtered through 0.22 μm filters so that no subsequent sterilisation is needed. Alternatively, established methods such as the use of moist heat (autoclave), ethylene oxide or gamma irradiation may be used.

The final product will preferably be prepared as a powder which will be reconstituted by the addition of sterile water for injection of sterile saline and then administered by intravenous injection. The powder may contain a suitable wetting agent such as Poloxamer 188 to aid redispersion, if needed.

CLAIMS

1. A process for preparing gas-containing microcapsules comprising forming microcapsules from a water-dispersible material, the microcapsules containing a liquid or solid core, and removing at least some of the said liquid or solid to create a microcapsule containing a gas.

2. A process according to Claim 1 wherein the water-dispersible material is water-soluble.

3. A process according to Claim 2 wherein the microcapsule walls are formed from water-soluble proteinaceous material and are subsequently made water-insoluble.

4. A process according to Claim 3 wherein the proteinaceous material is albumin.

5. A process according to any other of the preceding claims wherein the said core is a water-immiscible oil.

6. A process according to Claim 5 wherein the oil is relatively volatile and is removed from the oil-filled capsules by evaporation.

7. A process according to any other of the preceding claims wherein the microcapsules are formed by simple coacervation.

8. A process according to any one of Claims 1 to 6 wherein the microcapsules are formed by complex coacervation.

9. A process according to any one of Claims 1 to 6 wherein the microcapsules are formed by the process known as minimisation of solubility at isoelectric point.

10. A process according to any one of Claims 1 to 6 wherein the microcapsules are formed by a double-emulsion process.

11. A process according to any one of the preceding claims further comprising separating the gas-filled microcapsules from any liquid medium and freeze-drying the micro-capsules.

12. Microcapsules prepared by or obtainable by a process according to any one of the preceding claims.

13. A gas-filled microcapsule for use in diagnostic procedures, the gas-filled microcapsule having been formed by forming a microcapsule around a solid or liquid core and removing at least part of the said solid or liquid core.

14. A microcapsule having a plurality of gas-filled chambers therein.

15. A method of forming a diagnostic image comprising adding the microcapsules of any one of Claims 12 to 14 to the bloodstream of a patient, reflecting ultrasonic waves off the micro-capsules as they pass through or lodge in an organ to be imaged, and forming an image from the reflected waves.

16. A pharmaceutical composition for administration to the body comprising gas-filled microcapsules according to any one of Claims 12 to 14.

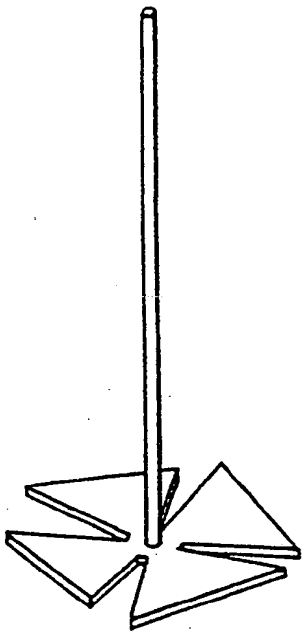

Fig. 1

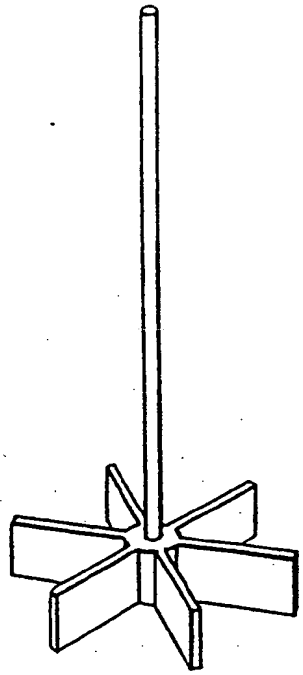

Fig. 2

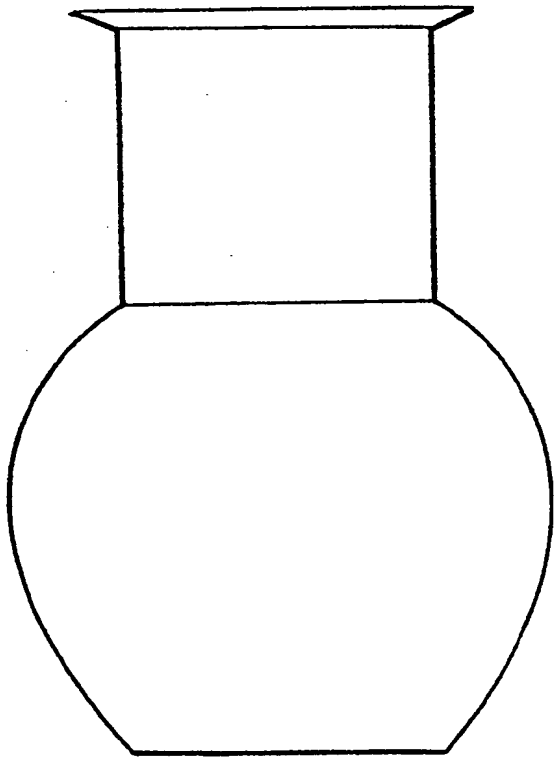

Fig. 3

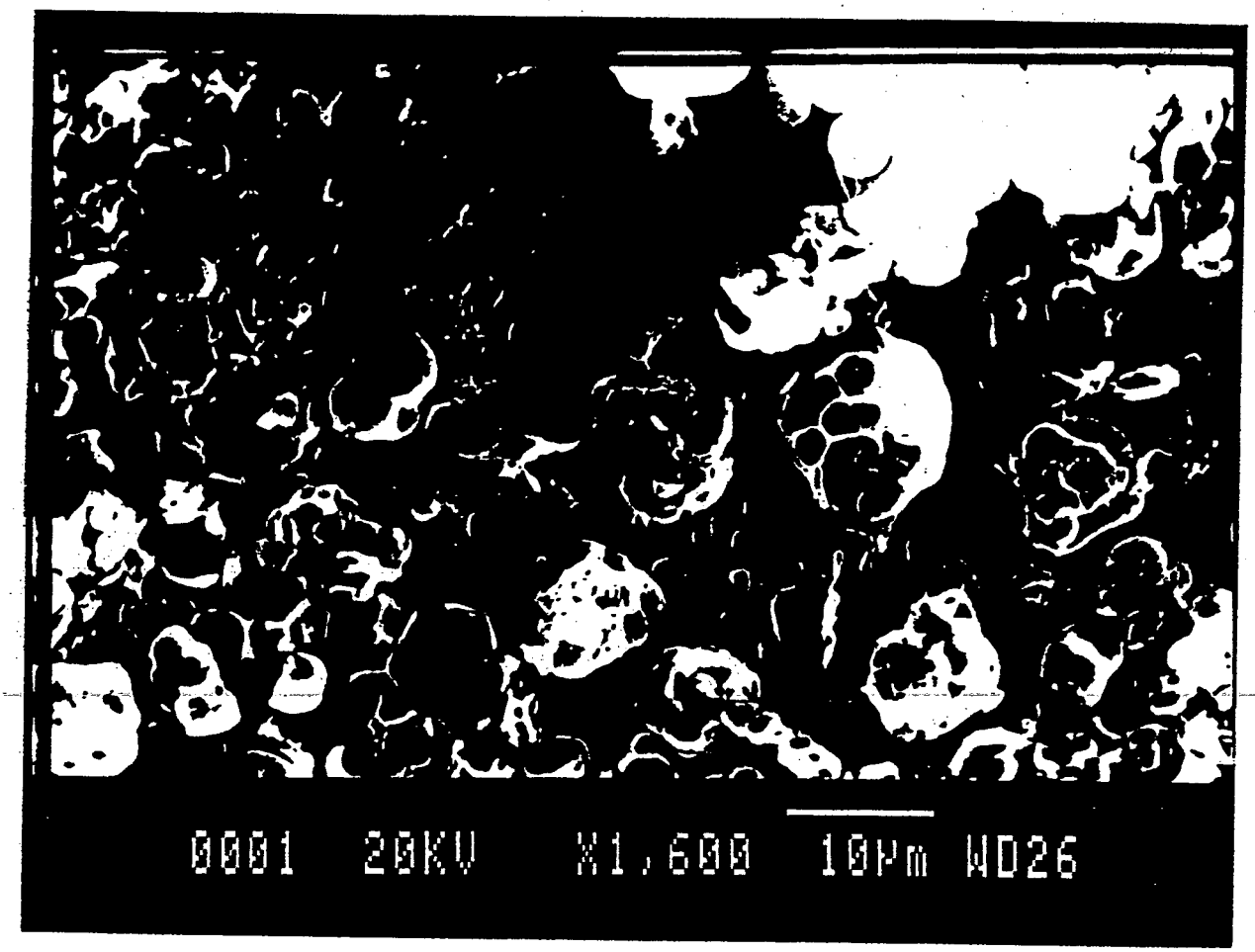

Figure 4

Figure 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00247

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 49/00, B 01 J 13/20, A 61 K 9/50

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K, B 01 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 3781230 (A.E. VASSILIADES et al.) 25 December 1973 see column 3, line 8 - column 4, lines 2,47 - column 5, lines 2,47 - column 6, line 8; column 10, lines 20-75; example 12 cited in the application	1-13,16
X	US, A, 4173488 (A.E. VASSILIADES et al.) 6 November 1979 see column 1, lines 12-17; column 1, line 40 - column 2, line 37; column 3, line 47 - column 4, line 37; example 1	1,2,5,6,11-13,16
Y	cited in the application	1-4,7-10
X	US, A, 4089800 (R.G.TEMPLE) 16 May 1978 see column 2, lines 5-25; column 7, line 28 - column 8, line 30; column 8, line 52 - column 9, line 6; claims 1-8	1,2,5,6,11-13,16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd May 1991	10. 07. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nicole Wanberg

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	cited in the application	1-4,7-10,13, 14,16

Y	EP, A, 0327490 (SCHERING A.G.) 9 August 1989 see column 2, line 11 - column 4, line 52; example 1 cited in the application	13,16

Y	A. Kondo: "Microcapsule Processing and Technology", 1979, Marcel Dekker, Inc. New York, US, see pages 18-20; page 61, table 7.1; pages 68,70,90-92,106-109,118-119 cited in the application	1-4,7-10,14

P,X	Database WPIL, Derwent 90-354613 & US, A, 4968562 (DELGADO J), 6 November 1990 see abstract	14

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 15, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1.(iv): methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9100247 SA 45006

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/06/91 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 3781230	25-12-73	BE-A-	739699	16-03-70
		CH-A-	502124	31-01-71
		DE-A-	1964424	03-08-72
		FR-A-	2026833	25-09-70
		GB-A-	1270408	12-04-72
		NL-A-	6915390	25-06-70
		SE-B-	375918	05-05-75
		US-A-	3822181	02-07-74
		US-A-	4173488	06-11-79
		US-A-	3919110	11-11-75
		US-A-	3585149	15-06-71
		US-A-	3707514	26-12-72
		US-A-	3720579	13-03-73
		US-A-	3752698	14-08-73
		US-A-	3779957	18-12-73
US-A- 4173488	06-11-79	BE-A-	775009	05-05-72
		CA-A-	988639	04-05-76
		CA-A-	979748	16-12-75
		DE-A-	2153358	15-06-72
		GB-A-	1365102	29-08-74
		GB-A-	1365101	29-08-74
		US-A-	3919110	11-11-75
		US-A-	3707514	26-12-72
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		FR-A-	2026833	25-09-70
		GB-A-	1270408	12-04-72
		NL-A-	6915390	25-06-70
		SE-B-	375918	05-05-75
		US-A-	3822181	02-07-74
		US-A-	3585149	15-06-71
		US-A-	3720579	13-03-73
		US-A-	3752698	14-08-73
		US-A-	3779957	18-12-73
		US-A-	3781230	25-12-73
US-A- 4089800	16-05-78	None		

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9100247 SA 45006

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0327490	09-08-89	DE-C-	3803971	07-09-89
		DE-A-	3803972	10-08-89
		AU-A-	3035189	25-08-89
		WO-A-	8906978	10-08-89
		EP-A-	0398935	28-11-90